

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. CERTIFICATE NUMBER: 84-R-0066
CUSTOMER NUMBER: 21162

FORM APPROVED
OMB NO. 0579-0036

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

Bolder Biopath, Inc.
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Boulder, CO 80301

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3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, or experimentation, or held for these purposes. Attach additional sheets if necessary)

FACILITY LOCATIONS (Sites) - See Attached Listing

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY (Attach additional sheets if necessary or use APHIS Form 7023A)

A. Animals Covered By The Animal Welfare Regulations	B. Number of animal being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals an for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for wh the use of appropriate anesthetic, analgesic, or tranquiliz drugs would have adversely affected the procedures, res or interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress in these animals and the reas such drugs were not used must be attached to this report	F. TOTAL NUMBER OF ANIMALS (COLUMNS C + D + E)
4. Dogs	/	/	/	/	/
5. Cats	/	/	/	/	/
6. Guinea Pigs	/	/	/	/	/
7. Hamsters	/	/	/	8	8
8. Rabbits	/	/	/	/	/
9. Non-human Primates	/	/	/	/	/
10. Sheep	/	/	/	/	/
11. Pigs	/	/	/	/	/
12. Other Farm Animals	/	/	/	/	/
13. Other Animals	/	/	/	/	/

ASSURANCE STATEMENTS

- 1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.
- 2) Each principal investigator has considered alternatives to painful procedures.
- 3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). A summary of all such exceptions is attached to this annual report. In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.
- 4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional Official)

(b)(6),(b)(7)(c)

(b)(6),(b)(7)(c)

L OFFICIAL (Type or Print)

DATE SIGNED

9/24/08

(which is obsolete.)

Column E Explanation

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

- Registration Number: 84-R-0066
- Number 8 of animals used in this study.
- Species (common name) Hamster of animals used in the study.
- Explain the procedure producing pain and/or distress.

Inflammatory bowel disease is a chronic debilitating inflammatory condition involving the distal small intestine and colon (enterocolitis). Antibiotic-induced colitis in hamsters (and humans) is associated with toxins produced by *Clostridium difficile* (*C. difficile*) and is characterized/confirmed clinically and histologically by hemorrhagic colitis (inflamed and edematous osceum) or through in vitro testing for *C. difficile* colony densities or *C. difficile* toxins. Hamsters will receive 1 dose (po, ip, sc) of a given antibiotic at the appropriate amount listed in the table below. Only one antibiotic will be used to induce enterocolitis per animal. Different antibiotics may be used on individual groups to compare the severity/mortality rates of said antibiotics.

Challenge Antibiotic	Dose (mg)*	Dose (mg/kg)*	Expected Mortality Rate
Cefazolin	60	800	90%
Cephaloridine	90	1125	90%
Cephalexin	75	1000	90%
Cefoxitin	2	25	90%
Cefamandole	60	800	90%
Cephadrine	75	1000	90%
Clindamycin	0.75	10	90%

*Minor adjustments may be made to the doses of antibiotics to counter any issues with antibiotic lot potencies.

These antibiotics have been shown to induce Enterocolitis, Pseudomembranous colitis and *Clostridium difficile* toxicity in hamsters over 2 to 10 days, with the gross observable end point being mortality. Hamsters may be treated sc, ip, po, or iv bolus or continuous infusion with test compounds in an attempt to inhibit the antibiotic-induced enterocolitis and subsequent deaths. Moribund animals will be humanely sacrificed when body weight loss is greater than or equal to 25% and/or if clinical signs of lethargy, *Clostridium difficile* toxicity and shock suggest that the animal will not recover.

- Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

Intestinal pain would call for narcotics, which would produce systemic effects that could increase the number of moribund animals. The negative effects of the narcotics may not benefit the animal, as there are no references suggesting that the animals suffer an inordinate amount of pain when bacterial infection is induced. Hamsters will lose weight as *Clostridium difficile* (*C. difficile*) colony sizes increase and toxin production begins. As the end point of the model is mortality, deaths will occur. If animals become moribund (inactive, unresponsive to touch stimuli, hypothermic) as a result of either the antibiotic or the test compound treatment, they will be humanely euthanized.

- What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHS, 9 CFR 113.102):

Agency _____ CFR _____

* These Hamsters Never Developed enterocolitis and were humanely sacrificed due to non-response.